

Biodiversity of arbuscular mycorrhizal fungi in different trees of madhupur forest, Bangladesh

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Abstract: Roots and rhizosphere soils of *Acacia auriculiformis* A. Cunn. ex Benth., *A. mangium* Wild., *Artocarpus heterophyllus* Lamk. C., *Dalbergia sissoo* Roxb. ex A. P. D., *Eucalyptus camaldulensis* Dehnn., *Hevea brasiliensis* (Wild. ex Juss) Muell. Arg., *Swietenia macrophylla* King. and *Tectona grandis* L. were collected from different locations of Madhupur forest area to study the biodiversity of Arbuscular Mycorrhizal (AM) fungal colonization and spore population. All the plants showed AM colonization. Out of eight selected plants, mycelial colonization was lowest in the roots of *A. heterophyllus* (22%) and the highest was in the roots of *H. brasiliensis* (78%). Mycelial intensity was observed poor (25%–77%) and moderate (23%–57%) in all plants species and abundant (11%–40%) was in most of the plant species. Vesicular colonization was observed in five plant species. The lowest was recorded in *E. camaldulensis* (4%) and the highest was in *H. brasiliensis* (21%). Poor (24%–56%), moderate (16%–100%) and abundant (11%–40%) type of vesicular intensity were observed. Arbuscular colonization was observed in three plants. The highest was in *A. mangium* (72%) and the lowest was in *S. macrophylla* (17%). Arbuscular intensity was recorded as poor (12%–44%), moderate (22%–100%) and abundant (4%–47%). The highest AM fungal spore population was in *A. auriculiformis* (714) and the lowest was in *D. sissoo* (102). Five AM fungal genera were recorded. *Glomus* was found to be dominant. A few spores remained unidentified. Significant correlation was observed between percent colonization and spore population. The results of the present study indicate the occurrence of AM fungi and the mycotrophism of the plants of Madhupur forest area and the applicability of AM technology in the forest management of Madhupur forest.

Key words: Biodiversity; Arbuscular Mycorrhizal fungi; Fungal colonization; Rhizosphere soils; Tree species.

CLC number: S718.81

Document code: A

Article ID: 1007-662X(2006)03-0201-05

Introduction

Madhupur Forest is in the district of Tangail, Bangladesh under AEZ-8 and it is situated in young Brahmaputra and Jamuna flood plain. The main soil texture of the forest area is loamy. It is in 'Madhupur Tracts' which is consisted of Plio-plietocene terraces in the central part of Bangladesh (Hassan, 1999) and ecologically it is tropical moist deciduous in nature (Das and Alam, 2001). The soils of Madhupur tract is acidic in nature (pH=5.2–5.5). Important trees and other plants were reported in the review of Hossain (2001). Although Sal (*Shorea robusta*) is the dominating species, different forest tree species are grown in plantations of forest areas under the Forest Department of Bangladesh Government.

Arbuscular Mycorrhizal (AM) fungi are now well practiced in the forestry management (Mukerji *et al.* 1996). They help in the establishment of the forest trees by increasing the volume of soil exploration and uptake of nutrients particularly Phosphorus, increasing disease resistance, improving water absorption, drought tolerance and thus accelerate the ability of the plant species to compete for resources, contributing to efficient recycling of nutrients particularly in acidic soils of tropical areas like Madhupur forests (Dhar and Mridha 2003). The biodiversity of AM colonization and AM fungi in different forest tree species were studied in India (Verma and Jamaluddin 1995; Kumar *et al.* 2000) and in Bangladesh (Dhar and Mridha 2003; Dhar *et al.*,

2005; Rahman *et al.* 2003). As such, the present study was carried out to observe the biodiversity of AM colonization, presence of AM fungal genera and the distribution of AM fungi in the rhizosphere soils of Madhupur forest areas.

Materials and methods

Roots and rhizosphere soils of different tree species (*Acacia auriculiformis* A. Cunn. ex Benth., *A. mangium* Wild., *Artocarpus heterophyllus* Lamk. C., *Dalbergia sissoo* Roxb. ex A. P. D., *Eucalyptus camaldulensis* Dehnn., *Hevea brasiliensis* (Wild. ex Juss) Muell. Arg., *Swietenia macrophylla* King. and *Tectona grandis* L.) were collected from different locations of Madhupur forest. Three replicated roots and rhizosphere soil samples were collected. Rhizosphere soils, fine and feeder roots were collected from 0–15 cm soil layer digging the soil with a soil corer. In the laboratory, roots were separated immediately from the soil and preserved in 5% formalin. Soil samples were studied earlier to avoid the damage of the spores in the rhizosphere soil. From each sample, 100g soil was taken in a bucket of 10-L capacity and 5-L of water was mixed with the soil. The soil was mixed well with water to make a soil-water suspension. The suspension was left for five minutes for settling down of insoluble and heavy particles. The suspension was passed through the ASTM-60, ASTM-100, ASTM-240 and ASTM-400 sieves gradually to extract the spores following by wet sieving and decanting method (Gerdemann and Nicolson 1963). The residues of the sieves were filtered with the Whatman filter paper No-1. Squares of intersecting gridlines were drawn earlier on the filter paper for easy counting of spore. After water filtration the paper was examined under the stereo-binocular microscope at 2.5×10 magnification and the number was recorded. Spores were separated on the basis of morphological characters and then they were ob-

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Received date: 2006-03-07; **Accepted date:** 2006-05-31

Responsible editor: Chai Ruihai

served under compound microscope mounting on PVLG and Melzer's reagent to determine the proper genus by following the established literature (Schenck and Perez, 1990). Percent population of individual genus was calculated by the following formula:

$$\% \text{ Genus} = \frac{\text{Number of individual genus}}{\text{Total number of spores}} \times 100$$

Preserved roots were washed with care to remove the formalin and chopped into 1 cm length for AM fungal structural analysis. They were cleaned and stained with aniline-blue following the method of Phillips and Hayman (1970). Ten segments were mounted on a slide (five segments each side) and examined under microscope at 10×10 magnification. Presence of mycelium, vesicles and arbuscules were recorded and analyzed for determining the structural colonization. The intensity of colonization was measured as poor, moderate and abundant (Dhar and Mridha 2003) colonized with each of the individual structure. Mycelial colonization was regarded as total AM colonization. Percent colonization was calculated by the following formula:

$$\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segment studied}} \times 100$$

Statistical analyses were accomplished by the SPSS-11.5 software. Simpson's diversity index and Shannon's Diversity index were calculated by following formulae:

$$\text{Simpson's Index (Ds)} = 1 - (\sum n_i^2 - N) / (N(N-1))$$

$$\text{Shannon's Index (Hs)} = \frac{C}{N} \{ (N \log_{10} N) - \sum n_i \log_{10} n_i \}$$

where 'C' = 3.321928 (constant used in converting log₁₀ to log₂), 'n_i' is the number of species in the 'ith' species and 'N' is the total number of individual (Simpson, 1949; Lloyd et al., 1968).

Results:

AM colonization:

Root samples of all the tree species showed AM colonization. Fig. 1 showed the total AM colonization in different tree species from Madhupur forest areas. Total AM colonization in different tree species varied significantly as indicated by the ANOVA. The range of colonization varied 22%–78%. The highest AM colonization was recorded in *H. brasiliensis* (78%) and it was followed by *T. grandis* (77%), *A. mangium* (73%), *A. auriculiformis*

(72%), *S. macrophylla* (48%), *E. camaldulensis* (36%) and *D. sissoo* (30%). The lowest was recorded in *A. heterophyllum* (22%). Structural colonization i.e. mycelial, vesicular and arbuscular colonization were recorded and the data have been presented in the Table 1. Mycelial colonization was recorded in all the tree species. The highest mycelial colonization was recorded in *H. brasiliensis* (78%) and the lowest was recorded in *A. heterophyllum* (22%). Vesicular colonization was recorded in four plants (8%–33%) such as *E. camaldulensis*, *H. brasiliensis*, *S. macrophylla* and *T. grandis*. Vesicular colonization varied significantly as indicated by ANOVA. The highest vesicular colonization was recorded in *T. grandis* (33%) and it was followed by *H. brasiliensis* (21%) and *S. macrophylla* (18%). The lowest was in *E. camaldulensis* (8%). *A. auriculiformis*, *A. mangium*, *A. heterophyllum* and *D. sissoo* showed no vesicular colonization. Arbuscular colonization was observed (17%–72%) in *A. mangium*, *H. brasiliensis* and *S. macrophylla*. The highest arbuscular colonization was recorded in *A. mangium* (72%) which was followed by *H. brasiliensis* (44%). The lowest was recorded in *S. macrophylla* (17%). Poor (25%–77%), moderate (23%–57%) and abundant (11%–40%) intensity of mycelial colonization varied considerably. Abundant intensity of mycelial colonization was observed in five plant species. Intensity of vesicular colonization was recorded poor (24%–56%), moderate (16%–50%) and abundant (34%–42%) respectively. Arbuscular colonization was observed in *A. mangium*, *H. brasiliensis*, and *T. grandis*. Poor, moderate and abundant intensity of arbuscular colonization was recorded 13%–14%, 40%–100% and 15%–47% respectively. Abundant intensity of arbuscular colonization was observed only in *A. mangium* (47%) and *H. brasiliensis* (15%).

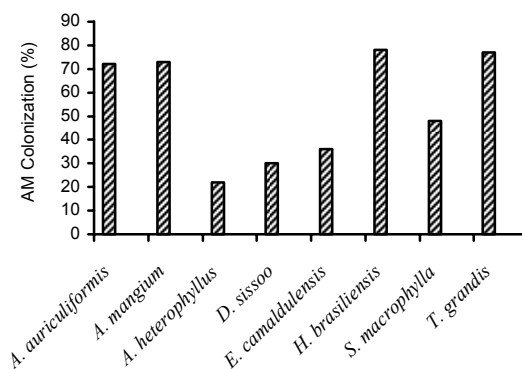


Fig. 1 Arbuscular mycorrhizal (AM) colonization in different tree species of Madhupur forest area

Table 1. Arbuscular mycorrhizal structural colonization in the roots of different forest trees of Madhupur

| Tree species | Total colonization (%) | | | Intensity of Colonization (%) | | | | | | | | | |
|--------------------------|------------------------|----------|------------|-------------------------------|----|----|----------|----|----|------------|-----|----|---|
| | | | | Mycelium | | | Vesicles | | | Arbuscules | | | |
| | Mycelium | Vesicles | Arbuscules | P | M | A | P | M | A | P | M | A | |
| <i>A. auriculiformis</i> | 72b* | 00e | 00d | 31 | 57 | 13 | - | - | - | - | - | - | - |
| <i>A. mangium</i> | 73b | 00e | 72a | 51 | 49 | - | - | - | - | 13 | 40 | 47 | |
| <i>A. heterophyllum</i> | 22f | 00e | 00d | 77 | 23 | - | - | - | - | - | - | - | - |
| <i>D. sissoo</i> | 30e | 00e | 00d | 53 | 47 | -- | - | - | - | - | - | - | - |
| <i>E. camaldulensis</i> | 36d | 8d | 00d | 59 | 39 | 11 | 50 | 50 | - | - | - | - | - |
| <i>H. brasiliensis</i> | 78a | 21b | 44b | 27 | 42 | 31 | 24 | 42 | 34 | 14 | 71 | 15 | |
| <i>S. macrophylla</i> | 48c | 18c | 17c | 25 | 35 | 40 | 56 | 44 | - | - | 100 | - | |
| <i>T. grandis</i> | 77a | 33a | 00d | 29 | 43 | 29 | 42 | 16 | 42 | - | - | - | - |

* Same letters showed no significant variation as indicated by DMRT at P<0.05.

AM fungal spore population:

The results of the soil assessment of different tree species have been presented in the Table 2. Total spore population of AM fungi in the rhizosphere soil of different forest trees varied significantly as indicated by the ANOVA. The highest spore population was recorded in the rhizosphere soil of *A. auriculiformis* (714) and it was followed by *E. camaldulensis* (471), *S. macrophylla* (440), *T. grandis* (423), *A. mangium* (330), *H. brasiliensis* (313) and *A. chaplasha* (211). The lowest was recorded in *D. sissoo* (102). There was significant and positive relation between AM colonization and spore population in the rhizosphere soil of the tree species of Madhupur forest area ($r^2=0.487$; $P<0.05$). *Glomus* was dominating among five genera detected in the rhizosphere soils of different forest trees of Madhupur forest area and it was followed by *Acaulospora*. There was significant variation in the percent population of *Glomus* as indicated by the ANOVA. The range of *Glomus* was 62%–94%. The highest was in *A. auriculiformis* (94%) and it was followed by *D. sissoo* (92%), *T. grandis* (88%), *H. brasiliensis* (85%). The lowest was in *E. camaldulensis* (67%) and *S. macrophylla* (67%). *Acaulospora* was observed in four plant species and the range of percent population was recorded as 7%–21%. The highest percent population of *Acaulospora* was recorded in *E. camaldulensis* (21%) and it was followed by *A. mangium* (19%) and *T. grandis* (8%). The lowest was recorded in *H. brasiliensis* (7%). *Entrophospora* was recorded in five tree species and the range was recorded as 2%–6% of the total population. The highest percent population of *Entrophospora* was recorded in *S. macrophylla* (6%) and it was followed by *A. heterophyllum* (5%), *D. sissoo* (4%) and *A. auriculiformis* (3%). The lowest was in *E. camaldulensis* (2%). *Gigaspora* was observed in six tree species and the

range was recorded 3%–22% of the total population. The highest was in *S. macrophylla* (22%) and it was followed by *A. heterophyllum* (12%), *A. mangium* (5%) and *T. grandis* (4%). The lowest was in *A. auriculiformis* (3%) and *H. brasiliensis* (3%). *Scutellospora* was observed in three four species and the range was recorded 2%–4%. The highest was in *E. camaldulensis* (4%) and *H. brasiliensis* (4%) and the lowest was in *D. sissoo* (2%) and *S. macrophylla* (2%). Simpson's diversity index (Ds) and Shannon's diversity index (Hs) in different tree species have been presented in the Fig. 2. The index value of "Ds" and "Hs" varied from 0.113–0.503 and 0.382–1.397 respectively. The highest "Ds" and "Hs" were observed in *E. camaldulensis* (0.503 and 1.397) and the lowest "Ds" and "Hs" were in *A. auriculiformis* (0.113 and 0.382).

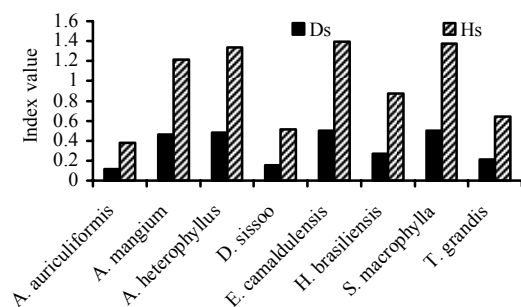


Fig. 2 Simpson's diversity index (Ds) and Shannon-Weiner diversity index (Hs) in the rhizosphere soils of different trees of Madhupur forest area

Table 2. Biodiversity of arbuscular mycorrhizal fungi in the rhizosphere soil of different tree species in Madhupur forest area

| Tree species | Total spore population | <i>Glomus</i> (%) | <i>Sclerocystis</i> (%) | <i>Acaulospora</i> (%) | <i>Entrophospora</i> (%) | <i>Gigaspora</i> (%) | <i>Scutellospora</i> (%) | Unidentified (%) |
|--------------------------|------------------------|-------------------|-------------------------|------------------------|--------------------------|----------------------|--------------------------|------------------|
| <i>A. auriculiformis</i> | 714a* | 94a | - | - | 3 | 3 | - | - |
| <i>A. mangium</i> | 330e | 71e | - | 19 | - | 5 | - | 5 |
| <i>A. heterophyllum</i> | 211g | 70g | - | - | 5 | 12 | - | 13 |
| <i>D. sissoo</i> | 102h | 92b | - | - | 4 | - | 2 | 2 |
| <i>E. camaldulensis</i> | 471b | 67f | - | 21 | 2 | - | 4 | 6 |
| <i>H. brasiliensis</i> | 313f | 85d | - | 7 | - | 3 | 4 | 1 |
| <i>S. macrophylla</i> | 440c | 67f | -- | -- | 6 | 22 | 2 | 3 |
| <i>T. grandis</i> | 423d | 88c | - | 8 | - | 4 | - | - |

*Different letters showed significant variations as indicated by DMRT at $P<0.05$.

Discussion

All the plant species from Madhupur forest areas showed AM colonization and spore population in their respective rhizosphere soils. Arbuscular mycorrhizal colonization and spore population varied significantly in different tree species as showed by the ANOVA. This study confirmed the widespread occurrence of arbuscular mycorrhizal fungi in the soils of Madhupur forest areas. The variation in the percentage of colonization in the roots and AM fungal spore population in the rhizosphere soils of different plantation forest trees recorded in the present study might explain that the plant species with regard to mycorrhizal colonization had a narrow to broad range of colonization. The present results of the study are in consistent with the reports of many authors (Muthukumar and Udaiyan, 2000; Ragupathy and Mahadevan 1993; Sharma *et al.* 1986). Onguene and Kuyper (2001)

reported the variation in AM colonization in different trees from the rain forest of South Cameroon. The variation in percent root colonization has been reported to be effected by seasonal sporulation, seasonal variation in development of host plants (Sutton and Barron 1972) and the nutrient availability in the soils (Louis and Lim 1987). This variation may be the results of variable host susceptibility (Mehrotra 1998), diverse type of AM fungi in the rhizosphere soils of individual plant species, host efficiency in soil resource capture and utilization (Koide, 1991; Clark and Zeto, 2000), soil types and quality (Raman and Gopinathan, 1992) and other edapho-climatic factors (Abbott and Robson, 1991). The involvement of intercellular or intracellular mycorrhizal associations or association of more than one mycorrhizal fungus with single host tree might be attributed to their physiological, ecological, and genetical variability (Sharma *et al.* 1986).

The higher concentration of soil nutrients particularly P could inhibit the root colonization n. The fact was that nutrient avail-

ability had a much stronger effect on the root architecture and in low-resource environment; root system physiology adapted to maximize the nutrient uptake capacity and the role of the AM fungi was to increase the uptake capacity of the active zone (see Cruz *et al.* 2004). The nutrient status of the forestlands of Madhupur forest areas might be different in various locations and the variation in AM colonization could be attributed as such. Several factors including seasonal sporulation, seasonal development of the host plants, seasonal nutrient availability, etc might have direct or indirect influence on the AM colonization (Fontenla, 1998). Soil disturbance has also an important effect on the root colonization. Alexander *et al.* (1992) reported that in the undisturbed ecosystems the rate of root colonization was higher. If the host plant had adequate resources or if the fungus was not compatible to the host plant the plant would not respond to the symbiosis. In the soils where nutrient deficiency became limiting factor, the host plant responded to the symbiosis of arbuscular mycorrhiza (Bhatia *et al.* 1996). Organic matter which serves as a nutrient sink for the plants could also regulate the intensity of mycorrhiza (Bhatia *et al.* 1996).

The AM fungal spore number recorded in the present study is greater than the previously reported number from tropics (Al-Garni and Daft, 1990; Jasper *et al.* 1990; Khan, 1974; Neeraj *et al.* 1991; Parvathi *et al.* 1984) which contradicts the view that perennial ecosystems contain fewer spores than fields subjected to annual disturbance (Hayman 1982). The features favoring the higher population may either be the conducive to edaphic conditions for sporulation like low nutrient status, high aeration and optimum moisture or the undisturbed conditions of the soils which allowed sufficient time for the build up of mycorrhizal spores (Chulan and Omar 1991). The spore number varied significantly within and between sites in the present observation. Spore population is affected by a wide range of soil, climatic, fungal and host factors (Anderson *et al.* 1983; Howeler *et al.* 1987). The observation of the present study indicates the variation of colonization irrespective of the spore number that supports the previous reports (Alexander *et al.* 1992; Alexander 1989; Janos 1983). AM fungal distribution, AM colonization and mycorrhizal efficiency might be influenced by soil pH, soil humidity level, etc (Abbott and Robson 1991; Moreira-Souza *et al.* 2003). Sharma *et al.* (1986) reported that the occurrence of AM species in the subtropical forest ecosystems of Meghalaya (India), are controlled to a great degree by the soil pH, organic matter, moisture and nutrients status of forest floor. The soil pH is known to control the availability of nutrients from the soils to plants thereby regulating the status of mycorrhiza (Baylis, 1967). Moreira-Souza *et al.* (2003) also reported higher spore population in their study. In an undisturbed ecosystem, higher spore population was quite natural as AM fungal spores tended to be higher and the population diversity was higher in native undisturbed forests than the disturbed and replanted areas (Moriera-souza *et al.* 2003; Sieverding 1991). It is clear from the report of Moreira Souza *et al.* (2003) that different soil pH determined the distribution of AM fungal species. The genetic diversity of AM fungi might be responsible to the variation in their pattern of production and colonization.

Patterns of spore production, spore quantity etc are closely related to the plant phenology, root phenology and root production (Brundrett 1991). Every life history of a mycorrhizal fungus is subjected to be influenced by plant roots. Spore germination, germination rate, direction of germ tubes hyphal branching recognition of the host root penetration establishment intensity of

colonization growth of hyphae into soils and sporulation of the AM fungi were reported to be affected by the plant roots (Bhatia *et al.* 1996). Different types of organic chemicals, volatile compounds were produced by the roots of different plants. Different volatile compounds like organic acids, alcohol etc could contribute to the activity and affect the life cycles of the AM fungi in the natural ecosystems. Thick root mats with greater number of fine roots, successful competition of mycorrhizal fungi with other fungi, seasons, soil moisture, soil type and nutrient level in the rhizosphere soils were reported to keep important role on the numbers of spores, activity of the spores, etc. (see Bhatia *et al.* 1996). Significant relationship was observed AM colonization and spore population ($r=0.487$ at $P<0.05$). Many researchers found positive relationship between AM colonization and spore population. Louis and Lim (1987) found high root colonization followed high number of spores in surrounding soils while Fontenla *et al.* (1998) found low frequency of colonization when the number of spore was high and vice versa. Incidence of arbuscules was reported to be moisture related. Many authors reported no significant relationship between AM colonization and spore population (see Dhar and Mridha 2003). It might be due to the different gradients by soil and the strong effect of plant factors on the formation, function and adaptation of the fungus to the respective soil conditions.

Out of six established genera, five genera were isolated from the rhizosphere soils of different forest tree plants under study. *Glomus* was the most distributed genera. *Glomus* sporulated abundantly regardless of tree species and sites selected. Sharma *et al.* (1986) also reported the dominance of the *Glomus*. They described the wider adaptation of the taxon in varied soil conditions. The sporulation pattern of *Glomus* might bring about the dominance of the taxon. Spores of *Glomus* are grown in cluster and sporulate more frequently while other like *Gigaspora*, *Scutellospora* etc sporulated singly. Thus, less population of Gigasporineae might be quite expectable. Variation of diversity indices (Ds, Hs) was observed in the present study. Different life duration of the different host plants had been reported to be the controlling factors of the species composition of AM fungi (see Muthukumar and Udaiyan 2000), thus the variation of diversity indices might be resulted in. There was also great diversity of mycorrhizal fungi often associated with the same plant (Allen and Boosalis 1983). Disturbance might also responsible for distribution of AM fungi (Jasper *et al.* 1989). Climatic seasons seem to be more influential on distribution and abundance of mycorrhizal spores. Mycorrhization of forest plants have recently been considered as the substitute of chemical fertilization regarding environment pollution and disease control for better management of tropical forests. More studies are being emphasized to select the suitable indigenous AM fungal strains for the establishment and managing the forests and to make the foresters and people conscious about the role of mycorrhiza as a tools to maintain and manage the forests environmentally friendly.

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